

The Hygiene Hypothesis in the Age of the Microbiome

Markus J. Ege

Dr. von Hauner Children's Hospital, Ludwig Maximilians University Munich and Comprehensive Pneumology Centre Munich, German Centre for Lung Research, Munich, Germany

ORCID ID: 0000-0001-6643-3923 (M.J.E.).

Abstract

The original version of the hygiene hypothesis suggested that infections transmitted early in life by “unhygienic contact” prevented allergies. Examples were endemic fecal–oral infections by viral, bacterial, or protozoic pathogens, such as hepatitis A virus, *Helicobacter pylori*, or *Toxoplasma gondii*. Later, this concept also included microorganisms beyond pathogens, such as commensals and symbionts, and the hygiene hypothesis was extended to inflammatory diseases in general. An impressive illustration of the hygiene hypothesis was found in the consistent farm effect on asthma and allergies, which has partly been attributed to immunomodulatory properties of endotoxin as emitted by livestock. Assessment of environmental microorganisms by molecular techniques suggested an additional protective effect of microbial diversity on asthma beyond atopy. Whether microbial diversity stands for a higher probability to encounter protective clusters of microorganisms or whether it is a proxy of a balanced environmental

exposure remains elusive. Diversity of the mucosal microbiome of the upper airways probably reflects an undisturbed balance of beneficial microorganisms and pathogens, such as *Moraxella catarrhalis*, which has been associated with subsequent development of asthma and pneumonia. In addition, specific fermenters of plant fibers, such as the genera *Ruminococcus* and *Bacteroides*, have been implied in asthma protection through production of short-chain fatty acids, volatile substances with the capability to reduce T-helper cell type 2–mediated allergic airway inflammation. Evolutionary thinking may offer a key to understanding noncommunicable inflammatory diseases as delayed adaptation to a world of fast and profound environmental changes. Better adaptation may be fostered by growing insight into the interplay between man and microbiome and an adequate choice of the environmental exposure.

Keywords: microbiome; hygiene hypothesis; environment; secular trend; noncommunicable disease

(Received in original form February 15, 2017; accepted in final form June 8, 2017)

Correspondence and requests for reprints should be addressed to Markus J. Ege, M.D., M.P.H., Dr. von Hauner Children's Hospital, Ludwig Maximilians University Munich, Lindwurm Strasse 4, 80337 Munich, Germany. E-mail: mege@med.lmu.de

Ann Am Thorac Soc Vol 14, Supplement 5, pp S348–S353, Nov 2017

Copyright © 2017 by the American Thoracic Society

DOI: 10.1513/AnnalsATS.201702-139AW

Internet address: www.atsjournals.org

A Short History of the Hygiene Hypothesis

The hygiene hypothesis emerged as a tentative explanation to a massive shift in the human disease spectrum from infections to allergies (1). It borrowed its name from a hypothesis originally proposed in the 1980s to explain the appendicitis epidemic of the early 20th century by an immune system not adequately trained for infections (2). Against the then prevailing idea that viruses triggered allergies (3), David Strachan suggested in 1989 (4) that allergies might be prevented by viral infections transmitted by “unhygienic contact” to siblings early in life. This notion was supported by subsequent

studies revealing inverse associations between allergies and endemic infections by viral, bacterial, or protozoic pathogens, such as hepatitis A virus, *Helicobacter pylori*, or *Toxoplasma gondii*, which are mostly transmitted by the fecal–oral route (5).

In the late 1990s, the hygiene hypothesis gained momentum with the striking farm effect on asthma and allergies and its obvious relation to microbial exposure (6). At the same time, awareness of the asthma and allergy epidemics was growing, and the hygiene hypothesis offered an attractive explanation to these phenomena (7).

Finally, the emerging T-helper cell type (Th) 1/Th2 paradigm provided a

theoretical foundation for the hygiene hypothesis (8), and, with several amendments, the co-occurrence of Th1- and Th2-dominated diseases in the same individual and the simultaneous rise in prevalence of both conditions (9) could be integrated in a larger concept of pro- and antiinflammatory processes (10).

Similar to the Th1/Th2 paradigm, the hygiene hypothesis itself underwent several transformations. The “old friends” (11) and the “disappearing microbes” (12) hypotheses are essentially revisions of the hygiene hypothesis. With a somewhat different slant, they both highlight the role of evolutionary adaptation to

microorganisms, regardless of whether they are commensals, symbionts, or pathogens. In addition, both approaches also endeavor to extend the hygiene hypothesis to autoimmunity, inflammatory disorders, and even to some forms of cancer.

The Difficult Relationship Between Hygiene and the Hygiene Hypothesis

The original label “hygiene” caused some confusion, as hygiene was often interpreted in the sense of personal hygiene or household cleanliness, rather than vertical or horizontal transmission of infections. In the context of the “gut microbial deprivation” hypothesis (13), an aberrant colonization of the neonatal intestine, due to an overly hygienic lifestyle, was postulated (14). Obviously, environmental conditions impact on the colonization of the neonatal gut (15), but an overly hygienic lifestyle is not comparable to experiments performed in germ-free mice, which may have led to a misconception of “sterile homes.” Even aggressive cleaning cannot clear microorganisms persistently (16).

The PAULA study (Perinatale Asthma Umwelt Langzeit Allergie Studie [in German]) exactly addressed the question of household and personal cleanliness, and collected data on frequency and intensity of cleaning, individual habits of hygiene behavior, and attitudes toward dirt. Though cleanliness was well reflected by microbial compounds in indoor dust, it did not impact on development or avoidance of allergic diseases (17).

The Microbial Exposure Par Excellence: Farming and Endotoxin

The protective effect of growing up on a farm on asthma and allergies has long been confirmed in various studies all over the world (18). The farming environment is rich in microbial exposure, and the atopy-protective effect of endotoxin (LPSs) has been well established in the farm children studies (6, 19). More precisely, the effect was attributed to gram-negative rods, the main source of endotoxins (20). Targeting the 16S ribosomal RNA gene of bacteria revealed the species, *Acinetobacter lwoffii*, as a promising candidate among the gram-negative rods (21). This species was already known for its atopy-protective potential from pre- and postnatal mouse models of allergic airway inflammation (22, 23), and subsequently a role as an immunomodulatory commensal of the skin was proposed (24).

Recently, the endotoxin effect has impressively been replicated with dust samples from two agricultural communities in the United States that share many properties, such as a common genetic ancestry and a traditional lifestyle (25). A main difference between the two communities is the way of farming; the Hutterites run highly industrialized farms, whereas the Amish adhere to a traditional type of farming with few changes during the last two centuries. In a mouse model of allergic asthma, dust extracts from Amish homes prevented airway hyperresponsiveness, whereas extracts from Hutterite homes did not. This was mainly attributed to the high endotoxin levels in Amish homes, which exceeded those of

Hutterite homes by seven times. The effect of Amish dust extracts was abrogated in knockout mice deficient for key molecules of the innate immunity, such as myeloid differentiation primary response 88 and Toll-like receptor adaptor molecule 1.

Further molecules have been implied in the endotoxin effect: endotoxins from environmental exposure, as it occurs on farms, may induce the ubiquitin modifying enzyme, A20, and thereby suppress the activation of epithelial and dendritic cells of the airway mucosa (26). A20 also plays an important role in establishing tolerance toward Toll-like receptor (TLR) ligands and bacteria in the intestinal epithelium, where it is induced by bacterial colonization (27). This leads directly to another potential mechanism (i.e., the structure of LPSs [endotoxins] produced by the prevailing human gut bacteria). The number of acyl chains and their arrangement within the endotoxin molecules determines whether endotoxin stimulates or inhibits the activation of TLR4 and nuclear factor κ -light-chain-enhancer of activated B cells (28).

Similarly, the farm exposure elicits a profound and persistent effect on the immune system. When pregnant women were exposed to livestock, gene expression of receptors of the innate immunity, such as the endotoxin receptors, CD14 and TLR4, was increased in their offspring at school age (29). Moreover, the expression of these indirect markers of microbial exposures was related to the diversity of animal exposure in a dose-dependent manner (Figure 1). The feed, feces, and body surfaces of different animal species are colonized by rather distinct

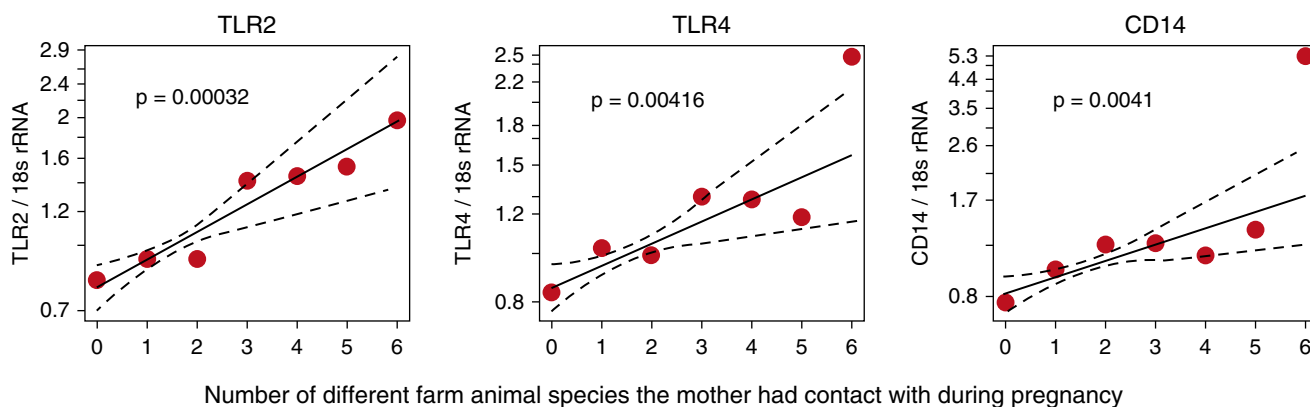


Figure 1. Diversity of animal contact as a potential proxy of microbial exposure. Reprinted from Reference 29 with permission from Elsevier. Solid lines are regression lines; dashed lines are 95% confidence intervals; red dots are gene expression values averaged over number of animal species. rRNA = ribosomal RNA; TLR = Toll-like receptor.

microorganisms. Hence, one might interpret the multitude of animal species as a proxy of microbial diversity.

Microbial Diversity and Asthma

Indeed, farm children are exposed to a higher diversity of environmental bacteria and fungi, as illustrated by Figure 2 (20). By contrast, the risk of childhood-onset asthma declined with increasing microbial diversity, independently of any other farm-related exposure. This finding lent support to the “microbial diversity hypothesis,” though the mechanisms behind this phenomenon still remain enigmatic. Most of all, it is unclear what diversity actually stands for. As the repertoire of receptors of the innate immunity is rather limited, the effects of diversity may involve other cascades beyond the redundantly organized innate immune system. A higher diversity definitively increases the stochastic probability of encountering advantageous microorganisms; alternatively, a high diversity might keep detrimental microorganisms at bay or may just be indicative of an established balance of microbial powers. In terms of the “biodiversity hypothesis” (30), one may also assume that microbial diversity reflects that of a superordinate system (i.e., the entire biosphere).

In contrast to the consistent effect on asthma, microbial diversity was not related to atopic sensitization and atopic disease

manifestation, such as hay fever (20). Likewise, atopy and asthma differ completely in their relation to endotoxin exposure. Whereas endotoxin protects from atopy, it increases the risk of nonatopic asthma and wheeze (6). Ultimately, these opposite effects challenge the hygiene hypothesis as a universal explanation to inflammatory disorders beyond atopy. Though both microbial diversity and endotoxin exposure are superficially related to microorganisms, they seem to reflect completely different phenomena (Figure 3). This paradox may be solved when considering the effects of microbial components and of microbial communities separately.

Beyond these paradoxes, two questions remain: (1) whether environmental bacteria impact on mucosal colonization; and (2) on which level the microbial exposure operates (i.e., by supplementing the host with metabolic functions, by producing immunomodulatory agents, or by horizontal gene transfer to mucosal bacteria).

Environmental and Mucosal Bacteria

The GABRIELA (Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community - Advanced Studies) study assessed the relation of the nasal

microbiome to environmental bacteria, as detected in mattress dust (31). The Bland-Altman plot given in Figure 4 illustrates a substantial agreement of α diversity from both sampling sources on an individual level. The average α diversity in mattress samples exceeds that of nasal swabs by about 200 operational taxonomic units. This difference is particularly pronounced in farm children, who generally score higher in bacterial diversity than their rural peers (Figure 4).

Taken together, these findings suggest transfer of environmental microorganisms to the mucosa of the upper airways. Deeper sequencing in larger populations will increase the resolution of microbial analyses and possibly identify specific effects of distinct microorganisms or microbial clusters beyond the currently evident associations with α diversity.

The farm effect has mostly been attributed to two major determinants (i.e., consumption of unprocessed milk and exposure to cows and straw) (32). As the stomachs of cows are essentially enormous fermenters, it is conceivable that farm children regularly inhale volatile substances, such as propionate and other short-chain fatty acids originating from fermentation of plant fibers (31). Propionate is produced by the genera *Bacteroides* and *Ruminococcus*, among others (33), which were more commonly found in mattress samples from farm

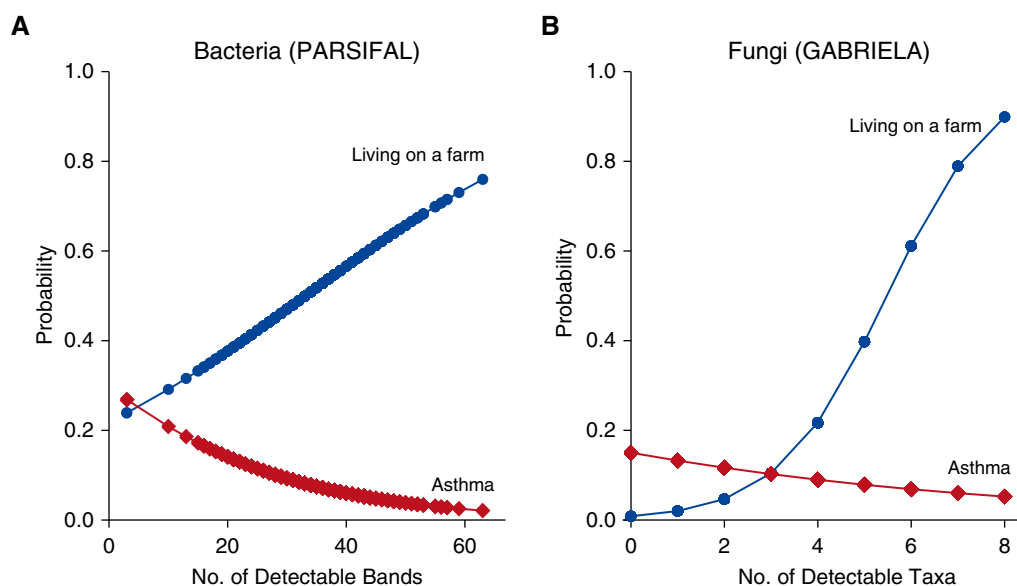


Figure 2. Diversity of environmental microorganisms and asthma. Reprinted from Reference 20 with permission from the Massachusetts Medical Society. GABRIELA = Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community - Advanced Studies; PARSIFAL = Prevention of Allergy — Risk Factors for Sensitization Related to Farming and Anthroposophic Lifestyle study.

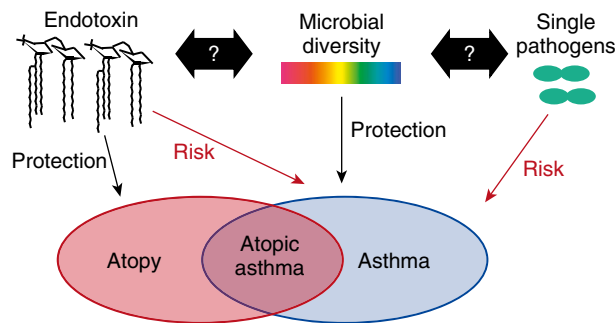


Figure 3. Differential effects of endotoxins, microbial diversity, and single pathogens. Endotoxin exposure protects from atopy, but increases the risk of nonatopic asthma. The risk of asthma in general is reduced by microbial diversity, but increased by single pathogens. How endotoxin exposure, microbial diversity, and single pathogens are interrelated is currently unclear.

children (31). A link to the farm effect on asthma seems likely, as short-chain fatty acids and, particularly, propionate have been shown to dampen Th2 cell-mediated allergic airway inflammation (34).

Microbial Virulence or Host Factors

In a different study region of the GABRIELA study, the relationship of nasal versus pharyngeal microbiota, and their impact on asthma, was explored (35). In the nasal samples, α diversity differed again between farm and reference children, and was reduced in children with asthma (Figure 5). In throat samples, however, α diversity was neither related to environmental exposure nor to asthma. This may indicate that the immune tissue of Waldeyer's ring rigorously constrains microbial colonization of the oropharynx, whereas the mucosa of the nasal cavity may be more reactive to external stimuli.

According to the united airway hypothesis, the villous epithelium of the nasal cavity may share more properties with that of the lower airways, and thus better reflect host-microbiome interactions at the site of asthma pathology (35). However, this remains mere speculation as long as the microbiomes of the upper and lower airways have not been compared systematically on a population level beyond case series (36).

The GABRIELA study also showed that the association of nasal α diversity with asthma was confounded by the presence of the genus *Moraxella*, possibly species *catarrhalis*, which has previously been cultured from hypopharyngeal swabs of neonates who subsequently developed asthma or pneumonia (37, 38). If the effect of nasal α diversity on asthma is largely explained by the absence of a pathogen, such as *Moraxella*, microbial diversity might just be an epiphenomenon of a well-established balance of microorganisms undisturbed by pathogens (Figure 3).

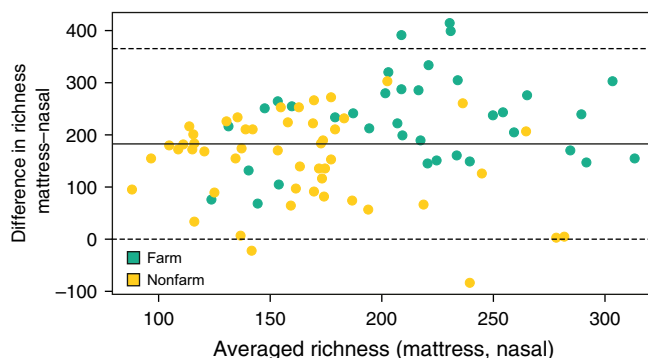


Figure 4. Agreement of α diversity between mattress and nasal samples. The solid line represents the average of the difference in richness between mattress dust and nasal swabs; the dashed lines mark ± 2 SDs. With the majority of single dots within the range of 4 SDs, the agreement between both samples sources is considerable. Reprinted from Reference 31 with permission from John Wiley and Sons.

The impact of *Moraxella* on asthma (37) seems to be unspecific, as it equally affects pneumonia, a rather distinct pathology (38). So, this generic effect points toward an underlying immune deficiency of the host rather than the virulence of a specific pathogen (39). In addition, the detection of pathogens in otherwise healthy subjects advocates against the virulence hypothesis, unless virulence might be enhanced by gene transfer from exogenous microorganisms in affected individuals.

Microbial diversity has also been implied in the relationship of gut microbiota and asthma (13, 40) following the gut-lung axis, a postulated communication based on observational changes in gut microbiome affecting lung immunity (41). Though preliminary analyses indicated profound imprinting of the environmental exposure on the diversity and composition of the gut microbiome of farm children, the gut microbiome is unlikely to mediate the farm effect on asthma (M. Depner and M.J.E., unpublished data).

Evolution in a Nutshell

The age of the microbiome has also promoted evolutionary thinking. The idea of coevolution of man and microbiome has finally replaced the concept of struggle between pathogen and host, which was more a mirror of human warfare than a biological explanation. Ultimately, microorganisms are neither friends nor foe, nor are they missing; and we do not live in a sterile world with too much hygiene. We are just in an era of rapid changes, which again will trigger evolutionary adaptation to an altered balance between man and microbiome. In an evolutionary diction, the increase in noncommunicable diseases merely reflects an adaptation that lags behind in a world of fast and profound environmental changes. With a growing insight into the interplay between man and microbiome, we might take over a more active role and enhance adaptation through an adequate choice of microbial exposure. Although complete restoration of a preindustrialized microbiome is an illusion, the comparison of populations with diverse microbial environments may offer some guidance.

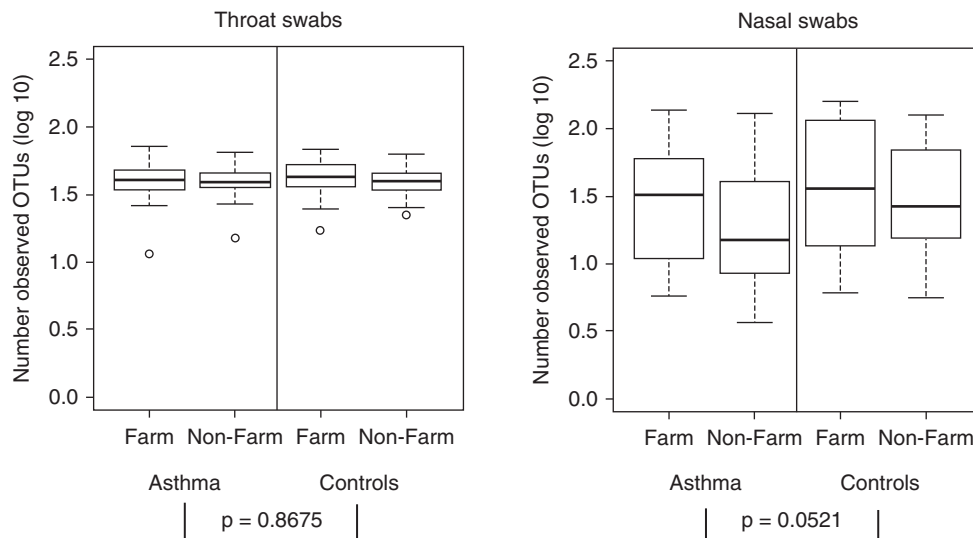


Figure 5. Richness in upper airway samples. Reprinted with modifications from Reference 35 with permission from Elsevier. Bold horizontal lines represent the medians, boxes the first and third quartiles, and whiskers the most extreme values within 1.5 times the interquartile range. Circles represent outliers. OTU = operational taxonomic unit.

A Plea for Diversity

The hygiene hypothesis has been a useful tool to approach various phenomena related to inadequate immune response. In all its flavors and by all its amendments, it added much to our understanding of the impact of microorganisms on human health. However, its limits should be acknowledged. The disparate effects of microbial diversity on asthma and

endotoxin on atopy illustrate that the hygiene hypothesis cannot serve as a generic explanation of any inflammatory noncommunicable disease. A more critical assessment of the various effects of microbial exposure on different disease phenotypes might reveal a more differentiated pattern.

Diversity proved to be an instructive concept for understanding the impact of the complex microbial exposure on asthma.

Likewise, it might prevent us from explaining multifaceted biologic effects by one and the same theory. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: This article is dedicated to Dr. Antje Legatzki (1975–2017), whose most recent work is summarized in the sections ENVIRONMENTAL AND MUCOSAL BACTERIA and MICROBIAL VIRULENCE OR HOST FACTORS.

References

- Ege M, Rompa S. The hygiene hypothesis of allergy and asthma. In: Ratcliffe MJH, editor. *Encyclopedia of immunobiology*. Oxford: Academic Press; 2016. pp. 328–335.
- Barker DJ. Acute appendicitis and dietary fibre: an alternative hypothesis. *Br Med J (Clin Res Ed)* 1985;290:1125–1127.
- Busse WW. The relationship between viral infections and onset of allergic diseases and asthma. *Clin Exp Allergy* 1989;19:1–9.
- Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259–1260.
- Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, Bonini S. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000;320:412–417.
- Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, et al.; Allergy and Endotoxin Study Team. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;347:869–877.
- Eder W, Ege MJ, von Mutius E. The asthma epidemic. *N Engl J Med* 2006;355:2226–2235.
- Holt PG. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. *Lancet* 1994;344:456–458.
- Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002;347:911–920.
- Jutel M, Akdis CA. T-cell subset regulation in atopy. *Curr Allergy Asthma Rep* 2011;11:139–145.
- Rook GA. Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. *Immunology* 2009;126:3–11.
- Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol* 2009;7:887–894.
- West CE, Renz H, Jenmalm MC, Kozyrskyj AL, Allen KJ, Vuillermin P, Prescott SL; in-FLAME Microbiome Interest Group. The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies. *J Allergy Clin Immunol* 2015;135:3–13. [Quiz, 14.]
- Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? *Allergy* 1998;53 (46 suppl):20–25.
- van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, Reijmerink NE, Dompeling E, van den Brandt PA, Ferreira I, Mommsers M, Thijs C. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. *J Allergy Clin Immunol* 2011;128:948–955.e1–3.
- Bloomfield SF, Stanwell-Smith R, Crevel RWR, Pickup J. Too clean, or not too clean: the hygiene hypothesis and home hygiene. *Clin Exp Allergy* 2006;36:402–425.
- Weber J, Illi S, Nowak D, Schierl R, Holst O, von Mutius E, Ege MJ. Asthma and the hygiene hypothesis: does cleanliness matter? *Am J Respir Crit Care Med* 2015;191:522–529.
- von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol* 2010;10:861–868.
- Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, Weiss G, Nyberg F, van Hage M, Pershagen G, et al.; PARSIFAL Study team.

- Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol* 2007;119:1140–1147.
- 20 Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, Heederik D, Piarroux R, von Mutius E; GABRIELA Transregio 22 Study Group. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* 2011;364:701–709.
 - 21 Ege MJ, Mayer M, Schwaiger K, Mattes J, Pershagen G, van Hage M, Scheynius A, Bauer J, von Mutius E. Environmental bacteria and childhood asthma. *Allergy* 2012;67:1565–1571.
 - 22 Debarry J, Garn H, Hanuszkiewicz A, Dickgreber N, Blümer N, von Mutius E, Bufe A, Gatermann S, Renz H, Holst O, et al. *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. *J Allergy Clin Immunol* 2007;119:1514–1521.
 - 23 Conrad ML, Ferstl R, Teich R, Brand S, Blümer N, Yildirim AO, Patrascan CC, Hanuszkiewicz A, Akira S, Wagner H, et al. Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe *Acinetobacter lwoffii* F78. *J Exp Med* 2009;206:2869–2877.
 - 24 Fyhrquist N, Ruokolainen L, Suomalainen A, Lehtimäki S, Veckman V, Vendelin J, Karisola P, Lehto M, Savinko T, Jarva H, et al. *Acinetobacter* species in the skin microbiota protect against allergic sensitization and inflammation. *J Allergy Clin Immunol* 2014;134:1301–1309.e11.
 - 25 Stein MM, Hrusch CL, Gozdz J, Igartua C, Pivniouk V, Murray SE, Ledford JG, Marques dos Santos M, Anderson RL, Metwali N, et al. Innate immunity and asthma risk in Amish and Hutterite farm children. *N Engl J Med* 2016;375:411–421.
 - 26 Schuijs MJ, Willart MA, Vergote K, Gras D, Deswarte K, Ege MJ, Madeira FB, Beyaert R, van Loo G, Bracher F, et al. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. *Science* 2015;349:1106–1110.
 - 27 Wang J, Ouyang Y, Guner Y, Ford HR, Grishin AV. Ubiquitin-editing enzyme A20 promotes tolerance to lipopolysaccharide in enterocytes. *J Immunol* 2009;183:1384–1392.
 - 28 Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, Kolde R, Vlamakis H, Arthur TD, Hämäläinen AM, et al.; DIABIMMUNE Study Group. Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell* 2016;165:842–853.
 - 29 Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E, Schram-Bijkerk D, Brunekreef B, van Hage M, Scheynius A, et al.; Parsifal Study Team. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J Allergy Clin Immunol* 2006;117:817–823.
 - 30 von Hertzen L, Hanski I, Haahtela T. Natural immunity: biodiversity loss and inflammatory diseases are two global megatrends that might be related. *EMBO Rep* 2011;12:1089–1093.
 - 31 Birzele LT, Depner M, Ege MJ, Engel M, Kublik S, Bernau C, Loss GJ, Genuneit J, Horak E, Schloter M, et al. Environmental and mucosal microbiota and their role in childhood asthma. *Allergy* 2017;72:109–119.
 - 32 Illi S, Depner M, Genuneit J, Horak E, Loss G, Strunz-Lehner C, Buchele G, Boznanski A, Danielewicz H, Cullinan P, et al.; GABRIELA Study Group. Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies. *J Allergy Clin Immunol* 2012;129:1470–1477.e6.
 - 33 Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, Flint HJ, Louis P. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *ISME J* 2014;8:1323–1335.
 - 34 Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014;20:159–166.
 - 35 Depner M, Ege MJ, Cox MJ, Dwyer S, Walker AW, Birzele LT, Genuneit J, Horak E, Braun-Fahrlander C, Danielewicz H, et al. Bacterial microbiota of the upper respiratory tract and childhood asthma. *J Allergy Clin Immunol* 2017;139:826–834.e13.
 - 36 Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, Davies J, Ervine A, Poulter L, Pachter L, et al. Disordered microbial communities in asthmatic airways. *PLoS One* 2010;5:e8578.
 - 37 Bisgaard H, Hermansen MN, Buchvald F, Loland L, Halkjaer LB, Bønnelykke K, Brasholt M, Heltberg A, Vissing NH, Thorsen SV, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007;357:1487–1495.
 - 38 Vissing NH, Chawes BL, Bisgaard H. Increased risk of pneumonia and bronchiolitis after bacterial colonization of the airways as neonates. *Am J Respir Crit Care Med* 2013;188:1246–1252.
 - 39 Ege M, von Mutius E. Microbial airway colonization: a cause of asthma and pneumonia? *Am J Respir Crit Care Med* 2013;188:1188–1189.
 - 40 Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clin Exp Allergy* 2014;44:842–850.
 - 41 Legatzki A, Rösler B, von Mutius E. Microbiome diversity and asthma and allergy risk. *Curr Allergy Asthma Rep* 2014;14:466.